Antimicrobial Efficacy of Plant Essential Oils and Extracts against *Escherichia coli*

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ABSTRACT

The efficacies of 11 plant-derived antimicrobials were evaluated against *Escherichia coli in vitro* in solution at room temperature. These included lemongrass, cinnamon, and oregano essential oils and their active components (citral, cinnamaldehyde, and carvacrol, respectively). Allspice and clove bud oils and olive, green tea, and grape seed extracts were also studied. The efficacies of the antimicrobials were both concentration- and exposure time-dependent. The essential oils and their active components demonstrated statistically significant >5.0-$\log_{10}$ reductions within 1-10 minutes. The plant extracts were less effective; green tea and grape seed extracts required 24 hours before significant reductions were observed (1.93-$\log_{10}$ and 5.05-$\log_{10}$, respectively). Nevertheless, olive extract exhibited a reduction of ~5-$\log_{10}$ within 30 minutes. Most of these plant-derived compounds exhibited strong bactericidal activity and can potentially be applied as alternatives to chemicals for foods/food contact surfaces since they are Generally Recognized As Safe (GRAS) for human consumption. They may also be useful in applications in which other antimicrobials have reduced efficacy (e.g., in the presence of organics) or used with sensitive populations that are unable to tolerate exposure to harsher chemicals (e.g., elderly care facilities). These compounds could be used alone, in combination, or with fast-acting antimicrobials to provide a long-lasting residual.

Keywords: *Escherichia coli*, plant antimicrobials, plant essential oils, active components, plant extracts

INTRODUCTION

Volatile or essential oils are aromatic viscous liquids that are responsible for the fragrance of plants [1] and are obtained from various plant materials (e.g., flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots). Plant extracts are obtained from many of these same sources but are available in the form of a powder. Numerous plant essential oils and extracts are of particular interest since many have been shown to possess antibacterial, antiviral, antifungal, insecticidal, and antioxidant properties. [2-24]

Essential oils may have as many as 60 individual components [25-26] and therefore often have multiple effects on the bacterial cell. [3] They may cause deterioration of the cell wall [3, 27], damage to the cell membrane [28] and membrane proteins [29], increased membrane permeability and the leakage of cell contents [3, 28], coagulation of the cytoplasm [30], reduction of the proton motive force [29], inactivation of essential enzymes [1, 31-32], and disturbance of genetic material functionality. [31]

Essential oils and extracts often have a dominant antimicrobial or active component which may account for greater than 50% of the chemical composition. [3] For example, eugenol is the primary active component in clove bud oil (up to 85% of its composition) [33-34] and accounts for its antioxidant properties. [35] Likewise, citral can account for up to 85% of the composition of lemongrass oil [36], the carvacrol content of oregano oil can be as high as 85% [18], and the cinnamaldehyde content of cinnamon oil can be as high as 86%. [18]

Extracts of olive pulp, grape seeds, and green tea contain members of the phenolic family. Catechol and epicatechin are two forms of simple phenols whose mechanism of action include substrate deprivation and membrane disruption. [37-38] The high concentrations of epicatechin and catechin in grape seed extract and caffeic acid and epicatechin in green tea extracts account for their high antioxidant activities. [39]

Many of these plant compounds are Generally Recognized As Safe (GRAS) for human consumption. [12, 40-41] Oregano oil has been used to add flavor to foods including salad dressings, tomato sauces, and pizzas. [18] Lemongrass is widely used for food flavoring, as a fragrance component in perfumes, and for its analgesic and anti-inflammatory characteristics. [41-42] Cinnamon oil is also used as a flavoring agent in various foods. [43]

In the current study, we compared the antimicrobial efficacy of three essential oils, oregano oil, lemongrass oil, and cinnamon oil, with their primary active components (carvacrol, citral, and cinnamaldehyde, respectively) against *Escherichia coli in vitro*. In addition, we examined five other plant essential oils and extracts (clove flower bud oil, allspice oil, olive extract, green tea extract, and grape seed extract) for their antimicrobial efficacy against *Escherichia coli*.

MATERIALS AND METHODS

Preparation of *Escherichia coli* and Plant Antimicrobials

Escherichia coli strain 25922 was obtained from the American Type Culture Collection (ATCC;

Manassas, VA). Long-term stocks of the *E. coli* were stored at -80°C in fetal bovine serum and the bacterium was maintained on tryptic soy agar (TSA; Difco, Sparks, MD). Prior to the start of each experiment, an Erlenmeyer flask containing 100 mL of Tryptic Soy Broth (TSB; Difco, Sparks, MD) was inoculated with the organism and incubated on an orbital shaker (Model G33; New Brunswick Scientific, Edison, NJ) set to 300 revolutions per minute (rpm) at 37°C overnight. After incubation, the *E. coli* were pelleted via centrifugation (9,820 × *g*, 15 minutes, 20°C). The pelleted cells were washed twice to remove organics by resuspension in 100 mL of physiological saline (0.85% NaCl) followed by centrifugation as described previously. The final pellet was re-suspended in 10 mL of sterile phosphate buffered saline (PBS; pH7.4; Sigma-Aldrich, St. Louis, MO). The test bacterial suspensions were then prepared by adding small volumes of the concentrated bacterial suspension to 10 mL of sterile PBS, resulting in an optical turbidity (measured using a BIOLOG turbidimeter, Hayward, CA) equaling a McFarland number 0.5 optical density standard. This is equivalent to approximately 1.5×10^8 colony-forming units (CFU)/mL. This solution was then diluted further in sterile PBS to achieve the desired final test concentration (approximately 1.0×10^7 CFU/mL).

Oregano, cinnamon, lemongrass, allspice, and clove bud essential oils were obtained from Lhasa Karnak Herbal Co. (Berkley, CA). Citral (mixture of *cis* and *trans*, >96%), carvacrol (>98%), and cinnamaldehyde (93%) were purchased from Sigma-Aldrich (St. Louis, MO). Green tea polyphenols extract was obtained from LKT Laboratories, Inc. (St. Paul, MN), grape seed extract was obtained from Swanson Health Products (Fargo, ND), and olive extract was obtained from CreAgri, Inc. (Hayward, CA). The plant antimicrobials were diluted to the specific concentrations needed for the experiments (volume to volume or weight to volume; vol/vol or wt/vol) using sterile

PBS. Alcohol was not used to dilute the sometimes viscous antimicrobials because of the potential for alcohol to enhance the antimicrobial efficacy of the solutions.

Experimental Protocol

The plant extracts, essential oils, and their active components were each evaluated in separate experiments. Screening trials consisted of systematically examining each plant antimicrobial for efficacy against *E. coli*. The method consisted of identifying appropriate working concentrations of the antimicrobials that are able to cause significant reductions. Initially, the essential oils and their active components were evaluated at 0.1% and 1.0% (vol/vol) concentrations and the extracts at 1.0% and 2.0% (wt/vol). These concentrations were chosen based on our previous experience working with these plant antimicrobials. These concentrations were then adjusted up or down as needed based on the experimental results to determine the working concentrations that were rapidly effective against *E. coli.* A control with *E. coli* in PBS but no added antimicrobial was also included in each experiment. Purified stocks of the bacteria were added separately to the antimicrobial solutions (to a final concentration of ~1.0×10^7 CFU/mL) and the flasks were placed on an orbital shaker (Model G33; New Brunswick Scientific, Edison, NJ) at 300 rpm for the duration of the experiment. Experiments were performed in triplicate at room temperature (24°C) in 10-mL volumes of PBS (pH 7.4) in 50 mL polypropylene conical tubes (Becton Dickinson and Company, Franklin Lakes, NJ). At predetermined time intervals (typically between one and 30 minutes of exposure; up to 24 hours for some extracts), 100-µL samples were collected and placed in Dey Engley (D/E) neutralizing broth (Difco, Sparks, MD) at a ratio of 1:10. The D/E was included since simultaneous studies were being conducted with other antimicrobials that required

this neutralizer. D/E has been used in similar studies in the past with plant antimicrobials. [44] The D/E was also used to neutralize the plant antimicrobials via dilution. The samples were assayed immediately as described below.

Assay for Bacteria

The surviving bacteria were enumerated by performing 10-fold serial dilutions in sterile saline buffer solution (0.85% NaCl) and spread plating on duplicate plates of Eosin Methylene Blue Agar (EMB; Becton, Dickinson and Company, Sparks, MD). The plates were incubated for 18 to 24 hours at 37°C and the bacterial colonies counted.

Antimicrobial Neutralization Experiments

In order to verify that the antimicrobials were sufficiently neutralized by dilution in D/E, a series of neutralization tests were performed with all concentrations of each plant antimicrobial in which a PBS solution containing the desired concentration of the antimicrobial was placed into D/E (1 mL into 9 mL). The solution was mixed thoroughly and then approximately 1.0×10^7 CFU of *E. coli* were added. The solution was mixed thoroughly again and then allowed to sit for five minutes at room temperature. Ten-fold serial dilutions were assayed on EMB agar plates as described previously. If the antimicrobials had been completely neutralized, it was expected that there would be no reduction in *E. coli* numbers in comparison to the controls with PBS alone (no antimicrobials added).

Statistical Analyses

The data were log-transformed to ensure a normal distribution. Data were reported as the logarithmic reduction using the formula $\log_{10}(N_0 / N_t)$, where N_0 was the population of *E. coli* at time zero and N_t was the surviving population at time *t*. A Student's t-test was used to determine if there were significant differences between the control and the antimicrobial treatments (the reduction at each time exposure with the antimicrobial was compared to the control at the end of the experiment, typically after 30 minutes). Differences were considered statistically significant if the resultant *P* value was ≤ 0.05. Differences between the reductions observed between various concentrations for each antimicrobial were evaluated for statistical significance using a Student's t-test.

Differences between the reductions observed between separate experiments with different antimicrobials (e.g., lemongrass oil versus cinnamon oil) were also evaluated for statistical significance using a Student's t-test. To allow for statistical comparisons between the reductions observed between different plant antimicrobials in separate experiments, the average $\log_{10}$ reduction in each experiment for the controls (at the end of the experiment, typically after 30 minutes) was subtracted from the reductions reported for each sample exposed to an antimicrobial (for all time exposure intervals) in order to normalize the reductions. When this resulted in a negative value, a reduction of 0.0-$\log_{10}$ was substituted. These normalized data were used for the Student's *t*-tests. The differences between the reductions were not statistically compared once the numbers had fallen to below the limit of detection (<50 CFU/mL) for both tests because there is no way of accurately determining if these reductions were different.

RESULTS AND DISCUSSION

No reductions in *E. coli* numbers were observed in the neutralization tests with any of the plant antimicrobials in comparison to the PBS controls (with no antimicrobials). Therefore, the dilution of samples by 1:10 in D/E was confirmed to completely neutralize all of the antimicrobials at the experimental concentrations.

The reductions observed in the controls (no added antimicrobials) by the end of the experiment (either 30 minutes or 24 hours) ranged from no reduction to 0.58-$\log_{10}$ (Average=0.10-$\log_{10}$). These were subtracted from the reductions reported for each treated sample (Tables 1-6) to allow for direct statistical comparisons between experiments.

Comparison between Essential Oils and their Active Components

Tables 1 through 3 show the results for lemongrass oil, cinnamon oil, and oregano oil in comparison to their active components citral, cinnamaldehyde, and carvacrol, respectively. Lemongrass oil concentrations of 0.05% and 0.3% were both highly effective, with >4-$\log_{10}$ reductions in *E. coli* populations within 10 and five minutes of exposure, respectively. The higher concentration yielded statistically significant reductions ($P \leq 0.05$ in comparison to the control) more rapidly than the lower concentration. In addition, the reduction observed at five minutes with the 0.3% lemongrass oil (4.37-$\log_{10}$) was significantly greater ($P=0.00055$) than that observed with the 0.05% concentration (0.55-$\log_{10}$). The detection limit of the assay (<50 CFU/mL) was reached

within five and 10 minutes of exposure for the high and low concentrations, respectively (equivalent to >5.94 and >5.62 $\log_{10}$ reductions).

The reductions observed with 0.05% and 0.1% citral were also rapid, with statistical significance reached within five minutes of exposure for both. The 0.1% citral treatment yielded a significantly greater reduction in the *E. coli* population than the 0.05% citral at every time interval. Interestingly, the 0.05% citral, although more rapidly effective than the 0.05% lemongrass oil (with a significantly greater reduction than lemongrass oil after five minutes), did not yield reductions as great with longer time exposures. In fact, the reductions observed for the 0.05% lemongrass oil were significantly higher than the 0.05% citral for both the 20- and 30-minute exposure times. In contrast, the 0.1% citral was equally as effective (P=0.52) as the 0.3% lemongrass oil, yielding 4.13-$\log_{10}$ and 4.37-$\log_{10}$ reductions, respectively after five minutes of exposure, and reductions to below the detection limit thereafter (<50 CFU/mL or >5.53 and >5.94 $\log_{10}$ reductions, respectively).

The results for cinnamon oil and its active component cinnamaldehyde are shown in Table 2. Cinnamon oil was tested at concentrations of 0.8% and 1.25%. The 0.8% trial produced a significant reduction (P=0.000001) of 2.20-$\log_{10}$ within one minute of exposure. The numbers of surviving bacteria at all subsequent time intervals were below the detection limit of the assay (<50 CFU/mL; >6.15-$\log_{10}$ reduction). The number recovered following exposure to the 1.25% concentration was below the detection limit (>5.53-$\log_{10}$; P=8.6×10^{-10}) within one minute of exposure.

Cinnamaldehyde concentrations of 0.1% and 0.2% were evaluated. The 0.1% trial produced a significant reduction of 2.01-$\log_{10}$ after 15 minutes (P=0.019) in comparison to the control); the 0.2% trial produced a significant reduction (P=0.001) of 0.69-$\log_{10}$ within one minute of exposure. Although much lower concentrations of cinnamaldehyde were tested in comparison to the cinnamon oil, similar reductions were observed, albeit with longer exposure times. For instance, 0.2% cinnamaldehyde resulted in a 5.11-$\log_{10}$ reduction after 10 minutes, whereas 1.25% cinnamon oil exhibited a >5.53-$\log_{10}$ reduction after 1 minute.

The results for oregano oil and its primary active component carvacrol are shown in Table 3. Both were tested at a concentration of 0.02%. A significant reduction (P=0.009 in comparison to the control) of 0.48-$\log_{10}$ was observed within five minutes with the 0.02% carvacrol, whereas 20 minutes was required for the oregano oil (P=0.033; 0.72-$\log_{10}$ reduction). Greater reductions ($P\leq$0.05) were observed with the 0.02% carvacrol than with the oregano oil at each common time exposure. At higher concentrations of 0.05% and 0.04% for oregano oil and carvacrol, respectively, the *E. coli* populations were reduced to below the detection limit (>5.35-$\log_{10}$ or >5.67-$\log_{10}$) within five minutes of exposure.

Limited information regarding the efficacy of essential oils in comparison to their active components is available. Most studies tend to focus on either a specific essential oil or active component. In a few studies, crude essential oils have been found to be more effective than their individual components [45-46], suggesting additive or possibly synergistic effects between the components. In the current study, we examined the efficacy of three essential oils and their corresponding active components against *E. coli*. Our results demonstrated that two of the active

components, citral and carvacrol, were more effective, (often at lower concentrations) than their corresponding essential oils. Delaquis et al. [47] observed similar findings with individual fractions of cilantro and dill essential oils. In the current study, citral was more rapidly effective, yet did not produce reductions as great as lemongrass oil with longer exposure periods. These differences may be at least partially explained by the relative proportion of the active component found in the essential oil. For instance, the carvacrol content of oregano oil may be as high as 85% and the cinnamaldehyde content of cinnamon oil as high as 86%, depending on their geographical origin. [18] Lemongrass oil contains multiple components including citral (57.5%), citral diethylacetal (24.7%), limonene (6.4%), citral acetate (2.1%), myrcene (1.2%), and methyl heptenone (1.2%). [42]

Comparing the antimicrobial efficacies of plant extracts, essential oils, and their active components allows for the establishment of a catalog of effective options. The efficacy of each of the plant antimicrobials included in the current study appears to be both concentration- and exposure time-dependent. Therefore, both concentration and exposure time are key factors that should be considered prior to their use. Other factors such as cost, availability, and organoleptic (sensory) properties will all affect the usage of essential oils or their active components against pathogens in various applications. Essential oils, although usually not quite as effective as their active components, exhibited significant antimicrobial activity. They are usually far less expensive to produce than their active components since there are fewer extraction or purification steps. They may also have less pungent organoleptic properties. Essential oils may therefore be a more viable option for use as an antimicrobial product. Future strategies could include combining essential oils or their active components with other plant extracts or essential oils, or with chemical agents such

as chlorine. [48]

Efficacy of Other Plant Antimicrobials

The two higher concentrations of clove bud oil (0.5% and 1.0% vol/vol) were rapidly effective (Table 4), causing a significant reduction ($P \leq 0.05$) within one minute of exposure (populations below the detection limit; <50 CFU/mL, equivalent to >5.52-$\log_{10}$ and >5.45-$\log_{10}$ reductions, respectively). The 0.06% concentration was also fairly effective, resulting in a significant reduction ($P=0.007$) of 0.32-$\log_{10}$ in the *E. coli* population within five minutes; nonetheless, this concentration required 30 minutes of exposure to reduce the population to a level that was below the detection limit of the assay (<50 CFU/mL; >5.35-$\log_{10}$) in some of the replicate samples. The differences between the reductions observed with the two higher concentrations and the 0.06% concentration were statistically significant ($P \leq 0.05$) at all of the time exposures with the exception of 30 minutes (in which all had at least one of the replicate samples that was below the detection limit of the assay and thus, could not be directly compared).

In contrast, a two-fold increase in allspice oil from 0.5% to 1.0% resulted in a vast difference in antimicrobial efficacy (Table 5). No reductions were observed with the lower concentration within 30 minutes of exposure, whereas a highly significant reduction ($P=0.00002$ in comparison to both the control and the 0.5% treatment) of >5.52-$\log_{10}$ was found with the 1.0% treatment after one minute of exposure and every time point thereafter.

Clove bud and allspice oils and their active component, eugenol, have previously been shown to

be effective against several foodborne pathogens. [8, 44, 49-51] These oils were similarly effective in the current study, showing no survivors (>5.52-$\log_{10}$ reductions) in *E. coli* populations within one minute of exposure at low concentrations (0.5% and 1.0%, respectively).

The results for the experiments conducted with olive extract are shown in Table 6. The 1.0% and the 2.5% concentrations were both effective, yielding similar statistically significant ($P \leq 0.05$ in comparison to the control) $\log_{10}$ reductions in *E. coli* populations at the earlier exposure time intervals (i.e., 15 and 20 minutes). Nevertheless, the 2.5% treatment exhibited significantly greater reductions ($P \leq 0.05$) than the 1.0% treatment with longer exposure times (i.e., 25 and 30 minutes). Limited data is available regarding the antimicrobial efficacy of olive pulp extracts; nonetheless, with nearly a 5-log reduction observed within 30 minutes of exposure, this extract is also highly effective against *E. coli* and could be useful as an antimicrobial in various applications.

Green tea extract and grape seed extract (Table 7) were only tested at higher concentrations (6.0% and 5.0% wt/vol, respectively) and for longer exposure times (up to 24 hours) based on the results of preliminary studies (data not shown). Concentrations higher than this are difficult to dissolve in the PBS test solution. These two extracts were not nearly as effective as the other antimicrobials included in this study (significant differences of $P \leq 0.05$ when comparing reductions after ≤ five minutes of exposure versus hours of exposure to green tea and grape seed extracts). Nonetheless, they did produce significant reductions (1.93-$\log_{10}$, $P=0.011$ and >5.05-$\log_{10}$, $P=0.0008$, respectively) in the *E. coli* population after 24 hours of exposure. Grape seed extract was significantly more effective than the green tea extract ($P=0.00004$).

Previous studies with grape seed extract have demonstrated poor antibacterial efficacy [52]; however, in one study, grape seed extract was shown to be effective against Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) strains. [53] Multiple studies with plant antimicrobials have indicated that some are more effective against Gram-positive bacteria than they are against Gram-negative bacteria. [3, 34, 47] This might explain why both grape seed extract and green tea extract had limited efficacy against the Gram-negative *E. coli* in the current study, requiring 24 hours to achieve a significant reduction.

Statistical Comparison of Plant Antimicrobial Efficacy

The most effective concentrations of each antimicrobial were compared to determine their relative antimicrobial efficacy. These included 0.3% lemongrass oil, 0.1% citral, 0.2% cinnamaldehyde, 0.05% oregano oil, 0.04% carvacrol, 0.5% clove bud oil, 1.0% allspice oil, 2.5% olive extract, 6.0% green tea extract, and 5.0% grape seed extract. A concentration of 0.8% cinnamon oil was chosen for the statistical comparisons over the 1.25% cinnamon oil since this lower concentration was still highly effective (complete reduction of >6.15-$\log_{10}$ within five minutes).

Clove bud oil and allspice oil were the most rapidly effective antimicrobials tested, with >5.52-$\log_{10}$ reductions observed within one minute of exposure. This was significantly greater than the reductions observed with any of the other antimicrobials tested ($P \leq 0.05$). Cinnamon oil and cinnamaldehyde were also found to result in significantly greater reductions in the *E. coli* populations within one minute of exposure than lemongrass oil or its active component citral ($P \leq 0.05$). Cinnamon oil worked significantly better ($P=0.0036$) than oregano oil after one minute.

No differences were observed between the reductions for oregano oil and either lemongrass oil (P=0.34), citral (P=0.76), or cinnamaldehyde (P=0.30) after one minute.

After five minutes of exposure, several changes in efficacy were observed. Oregano oil was found to be more effective than lemongrass oil ($P=2.63\times10^{-5}$) and cinnamaldehyde (P=0.0043) and equally as effective as citral (P=0.057). Although cinnamon oil was still more effective than lemongrass oil and citral ($P=2.0\times10^{-8}$ and P=0.003, respectively), these two antimicrobials in turn were found to be significantly more effective than cinnamaldehyde (P=0.0194 and P=0.0447, respectively). Carvacrol, which had not been tested at a one-minute exposure time, was found to be more effective than lemongrass oil ($P=5.3\times10^{-8}$), citral (P=0.008), and cinnamaldehyde (P=0.0003) after five minutes.

All of the plant essential oils and their active components were more rapidly effective against *E. coli* than olive extract. For instance, the reductions observed after one minute with clove bud and allspice oils (>5.52-$\log_{10}$ each) were significantly greater than the reduction observed for olive extract after 30 minutes (4.81-$\log_{10}$) (P=0.0058). Cinnamon oil, oregano oil, and carvacrol were more effective after five minutes than olive extract after 30 minutes (≥5.35-$\log_{10}$ vs. 4.81-$\log_{10}$; $P\leq0.05$). Lemongrass oil was more effective within five minutes than olive extract within 25 minutes (4.37-$\log_{10}$ vs. 3.39-$\log_{10}$; P=0.003). And finally, citral and cinnamaldehyde were more effective within five minutes than olive extract within 20 minutes (4.13-$\log_{10}$ and 3.01-$\log_{10}$, respectively vs. 2.24-$\log_{10}$ for olive extract; P=0.0041 and P=0.033, respectively).

In general, the efficacy of the various plant extracts, essential oils, or their active components

against *E. coli* was found to occur in the following ranking (from the most effective to the least effective based on their rapidity of action and the results of the statistical comparisons):

Clove Bud Oil = Allspice Oil > Cinnamon Oil = Carvacrol > Oregano Oil > Lemongrass Oil > Citral > Cinnamaldehyde > Olive Extract >>> Grape Seed Extract > Green Tea Extract

With the exception of carvacrol, essential oils were more effective than the individual active components. The individual active components were in turn more effective than the plant extracts. A summary of the time required for each concentration of the plant-derived antimicrobials to achieve a 99% or 2.0-$\log_{10}$ reduction in *E. coli* is provided in Table 8.

CONCLUSION

In the current study, we tested several plant-derived compounds with antimicrobial activities that make them promising candidates for use as antimicrobials. This study provides information regarding the potential concentrations and/or exposure times that would be required to achieve a particular desired log reduction in *E. coli* using these plant-derived antimicrobials. An advantage that natural antimicrobials might have over many traditional antimicrobials such as chlorine is that they likely have a longer lasting residual efficacy. [54] In addition, they are GRAS compounds [3, 55-58] and may therefore be used in situations in which toxic antimicrobials could not. As such, probably the most obvious use for plant antimicrobials is as food sanitizers for meats and fresh fruits and vegetables throughout the various stages of production from the processing plant, to the grocery store, to the consumer household. Unlike many other antimicrobials such as chlorine, plant

antimicrobials are not sensitive to organic matter. [54] Therefore, they could potentially promote a longer shelf life for food products because of their longer lasting residual effect. [54] Even the green tea extract and the grape seed extract were fairly effective with long exposure times; therefore, these could be used in situations in which a rapid kill is not required. Or they could be used in combination with a rapidly effective antimicrobial to provide an additional long-lasting residual effect. Since these plant compounds are GRAS, a food sanitizer based upon these antimicrobials would only need to undergo minimal regulatory testing prior to its use.

One great challenge when employing plant essential oils/extracts as food sanitizers is the problem of compatibility with respect to odor and taste. Whether they are derived from fruits or vegetables, all have specific aromatic notes. It is important to take advantage of these aroma notes and pair oils/extracts with compatible food items, much like how wine and cheeses are paired. For example, green tea extract could be used with apples, lemons, and other fruits traditionally combined with hot and cold tea drinks. Grape seed extract could potentially be applied to grapes, strawberries, and other vine-based fruits. While further testing is needed to elucidate the best olfactory pairings, the potential combinations are abundant. Many essential oil source products such as garlic and oregano are staples of many cuisines worldwide. Additionally, spices such as cinnamon and allspice are commonly added to foods as flavor additives. An effort should also be made to use the lowest effective concentrations, which should then minimize the aromatic and sensory effects and also serve to lower costs. Future work could include the testing of combinations of these plant-derived antimicrobials for the control of pathogenic bacteria such as *E. coli* in foods as well as tests to determine the compatibility of these compounds with various food types and flavors.

Plant extracts, essential oils, or their active components could be used in a wide variety of other applications as well. For instance, they could potentially be used as preservatives in cosmetics and lotions, they could be used to coat textiles (e.g., antimicrobial grocery bags), added to laundry or dishwashing detergents, or used in antimicrobial surface coatings or in liquid or spray applications to disinfect fomites (inanimate surfaces). Since they are considered GRAS, they can provide a non-toxic alternative to harsh chemical disinfectants in applications in which such chemicals are undesirable. For instance, they could be used in venues with sensitive populations such as in elderly care facilities, hospital intensive care units, and day care centers or schools.

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REFERENCES

[1] Cowan, M.M. Plant products as antimicrobial agents. Clin. Microbiol. Rev. **1999**, *12*, 564-582. https://doi.org/10.1128/CMR.12.4.564

[2] Bright, K.R.; Gilling, D.H. Chapter 16. Natural virucidal compounds in foods. In Viruses in Foods, 2nd ed.; Goyal, S.M., Cannon, J.L., Eds.; Springer Science: New York, NY, 2016; pp 449-469. https://doi.org/10.1007/978-3-319-30723-7_16

[3] Burt, S. Essential oils: their antibacterial properties and potential applications in foods - a review. Int. J. Food Microbiol. **2004**, *94*, 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

[4] Callaway, T.R.; Carroll, J.A.; Arthington, J.D.; Pratt, C.; Edrington, T.S.; Anderson, R.C.; Galyean, M.L.; Ricke, S.C.; Crandall, P.; Nisbet, D.J. Citrus products decrease growth of *E. coli* O157:H7 and *Salmonella* Typhimurium in pure culture and in fermentation with mixed ruminal microorganisms *in vitro*. Foodborne Pathog. Dis. **2008**, *5*, 621–627. https://doi.org/10.1089/fpd.2008.0088

[5] Carson, C.F.; Hammer, K.A.; Riley, T.V. *Melaleuca alternifolia* (tea tree) oil: A review of antimicrobial and other medicinal properties. Clin. Microbiol. Rev. **2006**, *19*, 50–62. https://doi.org/10.1128/CMR.19.1.50-62.2006

[6] Didry, N.; Dubreuil, L.; Pinkas, M. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. Pharm. Acta. Helv. **1994**, *69*, 25–28. https://doi.org/10.1016/0031-6865(94)90027-2

[7] Elizaquível, P.; Azizkhani, M.; Aznar, R.; Sánchez, G. The effect of essential oils on norovirus surrogates. Food Control **2013**, *32*, 275–278. https://doi.org/10.1016/j.foodcont.2012.11.031

[8] Friedman, M.; Henika, P.R.; Mandrell, R.E. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. J. Food Protect. **2002**, *65*, 1545–1560. https://doi.org/10.4315/0362-028X-65.10.1545

[9] Gilling, D.H.; Kitajima, M.; Torrey, J.T.; Bright, K.R. Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. J. Appl. Microbiol. **2014**, *116*, 1149-1163. https://doi.org/10.1111/jam.12453

[10] Gilling, D.H.; Kitajima, M.; Torrey, J.T.; Bright, K.R. Antiviral efficacy and mechanisms of action of plant antimicrobials against murine norovirus. Appl. Environ. Microbiol. **2014**, *80*, 4898-4910. https://doi.org/10.1128/AEM.00402-14

[11] Hammer, K.A.; Carson, C.F.; Riley, T.V. *In vitro* activity of *Melaleuca alternifolia* (tea tree) oil against dermatophytes and other filamentous fungi. J. Antimicrob. Chemother. **2002**, *50*, 195–199. https://doi.org/10.1093/jac/dkf112

[12] Knowles, J.R.; Roller, S.; Murray, D.B.; Naidu, A.S. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium. Appl. Environ. Microbiol. **2005**, *71*, 797–803. https://doi.org/10.1128/AEM.71.2.797-803.2005

[13] Kordali, S.; Kotan, R.; Mavi, A.; Cakir, A.; Ala, A.; Yildirim, A. Determination of the

chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. J. Agric. Food Chem. **2005**, *53*, 9452-9458. https://doi.org/10.1021/jf0516538

[14] Li, D.; Baert, L.; Zhang, D.; Xia, M.; Zhong, W.; Van Coillie, E.; Xiang, J.; Uyttendaele, M. The effect of grape seed extract on human norovirus GII.4 and murine norovirus-1 in viral suspensions, on stainless steel discs, and in lettuce wash water. Appl. Environ. Microbiol. **2012**, *78*, 7572–7578. https://doi.org/10.1128/AEM.01987-12

[15] Peñalver, P.; Huerta, B.; Borge, C.; Astorga, R.; Romero, R.; Perea, A. Antimicrobial activity of five essential oils against origin strains of the *Enterobacteriaceae* family. APMIS. **2005**, *113*, 1–6. https://doi.org/10.1111/j.1600-0463.2005.apm1130101.x

[16] Pinto, E.; Pina-Vaz, C.; Salgueiro, L.; Gonçalves, M.J.; Costa-de-Oliveira, S.; Cavaleiro, C.; Palmeira, A.; Rodrigues, A.; Martinez-de-Oliveira, J. Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. J. Med. Microbiol. **2006**, *55*, 1367–1373. https://doi.org/10.1099/jmm.0.46443-0

[17] Ravishankar, S.; Zhu, L.; Law, B.; Joens, L.; Friedman, M. Plant-derived compounds inactivate antibiotic-resistant *Campylobacter jejuni* strains. J. Food Protect. **2008**, *71*, 1145–1149. https://doi.org/10.4315/0362-028X-71.6.1145

[18] Ravishankar, S.; Zhu, L.; Olsen, C.W.; McHugh, T.H.; Friedman, M. Edible apple film wraps containing plant antimicrobials inactivate foodborne pathogens on meat and poultry products. J. Food Sci. **2009**, *74*, M440-M445. https://doi.org/10.1111/j.1750-3841.2009.01320.x

[19] Ravishankar, S.; Zhu, L.; Reyna-Granados, J.; Law, B.; Joens, L.; Friedman, M. Carvacrol and cinnamaldehyde inactivate antibiotic-resistant *Salmonella enterica* in buffer and on celery and oysters. J. Food Protect. **2010**, *73*, 234-240. https://doi.org/10.4315/0362-028X-73.2.234

[20] Reichling, J.; Koch, C.; Stahl-Biskup, E.; Sojka, C.; Schnitzler, P. Virucidal activity of a *β*-triketone-rich essential oil of *Leptospermum scoparium* (manuka oil) against HSV-1 and HSV-2 in cell culture. Planta. Med. **2005**, *71*, 1123–1127. https://doi.org/10.1055/s-2005-873175

[21] Reichling, J.; Schnitzler, P.; Suschke, U.; Saller, R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties – an overview. Forsch. Komplementmed. **2009**, *16*, 79–90. https://doi.org/10.1159/000207196

[22] Su, X.; D'Souza, D.H. Grape seed extract for control of human enteric viruses. Appl. Environ. Microbiol. **2011**, *77*, 3982–3987. https://doi.org/10.1128/AEM.00193-11

[23] Su, X.; Sangster, M.Y.; D'Souza, D.H. Time-dependent effects of pomegranate juice and

pomegranate polyphenols on foodborne viral reduction. Foodborne Pathog. Dis. **2011**, *8*, 1177–1183. https://doi.org/10.1089/fpd.2011.0873

[24] Tait, S.; Salvati, A.L.; Desideri, N.; Fiore, L. Antiviral activity of substituted homoisoflavonoids on enteroviruses. Antiviral Res. **2006**, *72*, 252–255. https://doi.org/10.1016/j.antiviral.2006.07.003

[25] Russo, M.; Galletti, G.C.; Bocchini, P.; Carnacini, A. Essential oil chemical composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. hirtum (Link) Ietswaart): A preliminary evaluation of their use in chemotaxonomy by cluster analysis: 1. Inflorescences. J. Agric. Food Chem. **1998**, *46*, 3741– 3746. https://doi.org/10.1021/jf980087w

[26] Senatore, F. Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). J. Agric. Food Chem. **1996**, *44*, 1327– 1332. https://doi.org/10.1021/jf950508z

[27] Thoroski, J.; Blank, G.; Biliaderis, C. Eugenol induced inhibition of extracellular enzyme production by *Bacillus cereus.* J. Food Protect. **1989**, *52*, 399-403. https://doi.org/10.4315/0362-028X-52.6.399

[28] Ultee, A.; Bennik, M.H.J.; Moezelaar, R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ.

Microbiol. **2002**, *68*, 1561-1568. https://doi.org/10.1128/AEM.68.4.1561-1568.2002

[29] Ultee, A.; Kets, E.P.W.; Smid, E.J. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. **1999**, *65*, 4606-4610.

[30] Gustafson, J.E.; Liew, Y.C.; Chew, S.; Markham, J.L.; Bell, H.C.; Wyllie, S.G.; Warmington, J.R. Effects of tea tree oil on *Escherichia coli*. Lett. Appl. Microbiol. **1998**, *26*, 194-198. https://doi.org/10.1046/j.1472-765X.1998.00317.x

[31] Ayala-Zavala, J.F.; Del-Toro-Sánchez, L.; Alvarez-Parrilla, E.; González-Aguilar, G.A. High relative humidity in-package of fresh-cut fruits and vegetables: Advantage or disadvantage considering microbiological problems and antimicrobial delivering systems? J. Food Sci. **2008**, *73*, R41-R47. https://doi.org/10.1111/j.1750-3841.2008.00705.x

[32] Wendakoon, C.N.; Sakaguchi, M. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J. Food Protect. **1995**, *58*, 280-283. https://doi.org/10.4315/0362-028X-58.3.280

[33] Bauer, K.; Garbe, D.; Surburg, H. Common fragrance and flavor materials: Preparation, properties and uses; Wiley-VCH: Weinheim, Germany, 2001.

[34] Farag, R.S.; Daw, Z.Y.; Hewedi, F.M.; El-Baroty, G.S.A. Antimicrobial activity of some Egyptian spice essential oils. J. Food Protect. **1989**, *52*, 665–667.

https://doi.org/10.4315/0362-028X-52.9.665

[35] Ogata, M.; Hoshi, M.; Urano, S.; Endo, T. Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chem. Pharm. Bull. **2000**, *48*, 1467-1469. https://doi.org/10.1248/cpb.48.1467

[36] de Bona da Silva, C.; Guterres, S.S.; Weisheimer, V.; Schapoval, E.E.S. Antifungal activity of the lemongrass oil and citral against *Candida* spp. Braz. J. Infect. Dis. **2008**, *12*, 63–66. http://dx.doi.org/10.1590/S1413-86702008000100014

[37] Peres, M.T.; Delle Monache, F.; Cruz, A.B.; Pizzolatti, M.G.; Yunes, R.A. Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (*Euphorbiaceae*). J. Ethnopharmacol. **1997**, *56*, 223-226. https://doi.org/10.1016/S0378-8741(97)00039-1

[38] Toda, M.; Okubo, S.; Ikigai, H.; Suzuki, T.; Suzuki, Y.; Shimamura, T. The protective activity of tea against infection by *Vibrio cholerae* O1. J. Appl. Microbiol. **1991**, *70*, 109-112. https://doi.org/10.1111/j.1365-2672.1991.tb04435.x

[39] Rababah, T.M.; Hettiarachchy, N.S.; Horax, R. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. J. Agric. Food Chem. **2004**, *52*, 5183-5186. https://doi.org/10.1021/jf049645z

[40] Adams, T.B.; Cohen, S.M.; Doull, J.; Feron, V.J.; Goodman, J.I.; Marnett, L.J.; Munro, I.C.; Portoghese, P.S.; Smith, R.L.; Waddell, W.J.; et al. The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. Food Chem. Toxicol. **2004**, *42*, 157–185. https://doi.org/10.1016/j.fct.2003.08.021

[41] Ress, N.B.; Hailey, J.R.; Maronpot, R.R.; Bucher, J.R.; Travlos, G.S.; Haseman, J.K.; Orzech, D.P.; Johnson, J.D.; Hejtmancik, M.R. Toxicology and carcinogenesis studies of microencapsulated citral in rats and mice. Toxicol. Sci. **2003**, *71*, 198–206. https://doi.org/10.1093/toxsci/71.2.198

[42] Katsukawa, M.; Nakata, R.; Takizawa, Y.; Hori, K.; Takahashi, S.; Inoue, H. Citral, a component of lemongrass oil, activates PPARα and γ and suppresses COX-2 expression. Biochim. Biophys. Acta **2010**, *1801*, 1214-1220. https://doi.org/10.1016/j.bbalip.2010.07.004

[43] Friedman, M.; Kozukue, N.; Harden, L.A. Cinnamaldehyde content in foods determined by gas chromatography-mass spectrometry. J. Agric. Food Chem. **2000**, *48*, 5702–5709. https://doi.org/10.1021/jf000585g

[44] Mattson, T.E.; Johny, A.K.; Amalaradjou, M.A.R.; More, K.; Schreiber, D.T.; Patel, J.; Venkitanarayanan, K. Inactivation of *Salmonella* spp. on tomatoes by plant molecules. Int. J. Food Microbiol. **2011**, *144*, 464-468. https://doi.org/10.1016/j.ijfoodmicro.2010.10.035

[45] Lachowicz, K.J.; Jones, G.P.; Briggs, D.R.; Bienvenu, F.E.; Wan, J.; Wilcock, A.; Coventry, M.J. The synergistic preservative effects of the essential oils of sweet basil (*Ocimum basilicum* L.) against acid-tolerant food microflora. Lett. Appl. Microbiol. **1998**, *26*, 209-214. https://doi.org/10.1046/j.1472-765X.1998.00321.x

[46] Vardar-Unlü, G.; Candan, F.; Sökmen, A.; Daferera, D.; Polissiou, M.; Sökmen, M.; Dönmez, E.; Tepe, B. Antimicrobial and antitoxic activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. var. *pectinatus* (Lamiaceae). J. Agric. Food Chem. **2003**, *51*, 63-67. https://doi.org/10.1021/jf025753e

[47] Delaquis, P.J.; Stanich, K.; Girard, B.; Mazza, G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbiol. **2002**, *74*, 101–109. https://doi.org/10.1016/S0168-1605(01)00734-6

[48] Beltrán, D.; Selma, M.V.; Tudela, J.A.; Gil, M.L. Effect of different sanitizers on microbial and sensory quality of fresh-cut potato strips stored under modified atmosphere or vacuum packaging. Postharvest Biol. Technol. **2005**, *37*, 37-46. https://doi.org/10.1016/j.postharvbio.2005.02.010

[49] Friedman, M.; Henika, P.R.; Levin, C.E.; Mandrell, R.E. Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. J. Agric. Food Chem. **2004**, *52*, 6042-6048. https://doi.org/10.1021/jf0495340

[50] Du, W.X.; Olsen, C.W.; Avena-Bustillos, R.J.; McHugh, T.H.; Levin, C.E.; Mandrell, R.; Friedman, M. Antibacterial effects of allspice, garlic, and oregano essential oils in tomato films determined by overlay and vapor-film methods. J. Food Sci. **2009**, *74*, M390-M397. https://doi.org/10.1111/j.1750-3841.2009.01289.x

[51] Pérez-Conesa, D.; Cao, J.; Chen, L.; McLandsborough, L.; Weiss, J. Inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 biofilms by micelle-encapsulated eugenol and carvacrol. J. Food Protect. **2011**, *74*, 55-62. https://doi.org/10.4315/0362-028X.JFP-08-403

[52] Mayer, R.; Stecher, G.; Wuerzner, R.; Silva, R.C.; Sultana, T.; Trojer, L.; Feuerstein, I.; Krieg, C.; Abel, G.; Popp, M.; et al. Proanthocyandins: target compounds as antibacterial agents. J. Agric. Food Chem. **2008**, *56*, 6959-6966. https://doi.org/10.1021/jf800832r

[53] Al-Habib, A.; Al-Saleh, E.; Safer, A.M.; Afzal, M. Bactericidal effect of grape seed extract on methicillin-resistant *Staphylococcus aureus* (MRSA). J. Toxicol. Sci. **2010**, *35*, 357-364. https://doi.org/10.2131/jts.35.357

[54] Sapers, M.G. Chapter 16. Disinfection of contaminated produce with conventional washing and sanitizing technology. In The Produce Contamination Problem: Causes and Solutions; Sapers, G.M., Solomon, E.B., Matthews, K.R., Eds.; Academic Press: Maryland Heights, MO, 2009; pp 393-424. https://doi.org/10.1016/B978-0-12-374186-8.00016-1

[55] Baskaran, Y.; Periyasamy, V.; Venkatraman, A.C. Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. Toxicol. **2010**, *268*, 204–212. https://doi.org/10.1016/j.tox.2009.12.018

[56] Hsu, Y.W.; Tsai, C.F.; Chen, W.K.; Huang, C.F.; Yen, C.C. A subacute toxicity evaluation of green tea (*Camellia sinensis*) extract in mice. Food. Chem. Toxicol. **2011**, *49*, 2624-2630. https://doi.org/10.1016/j.fct.2011.07.007

[57] Kumar, A.; Shukla, R.; Singh, P.; Dubey, N.K. Chemical composition, antifungal and antiaflatoxigenic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial. Food Chem. Toxicol. **2010**, *48*, 539-543. https://doi.org/10.1016/j.fct.2009.11.028

[58] Soni, M.G.; Burdock, G.A.; Christian, M.S.; Bitler, C.M.; Crea, R. Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. Food Chem. Toxicol. **2006**, *44*, 903-915. https://doi.org/10.1016/j.fct.2006.01.008

Table 1. Reduction (Log_{10} ± standard deviation)* in the population of *Escherichia coli* after exposure to various concentrations (vol/vol) of lemongrass oil or its active component, citral in PBS.

Time (min)	Lemongrass Oil		Citral	
	0.05 %	0.3 %	0.05 %	0.1 %
1	0.02 ± 0.08	0.00 [c] ± 0.00	0.01 [b] ± 0.03	0.23 [bc] ± 0.11
5	0.55 [bc] ± 0.32	4.37 [ab] ± 0.01	1.47 [abc] ± 0.19	4.13 [ab] ± 0.54
10	4.79 [a] ± 1.44	> 5.94 [a] ± 0.00	2.58 [ab] ± 0.76	> 5.53 [ab] ± 0.00
20	> 5.62 [ac] ± 0.00	> 5.94 [a] ± 0.00	3.52 [abc] ± 0.35	> 5.53 [ab] ± 0.00
30	> 5.62 [ac] ± 0.00	> 5.94 [a] ± 0.00	4.30 [abc] ± 0.62	> 5.53 [ab] ± 0.00

* Inoculated with 2.1×10^7 CFU/mL, 4.3×10^7 CFU/mL, 1.7×10^7 CFU/mL, and 2.5×10^7 CFU/mL for the experiment with 0.05% lemongrass oil, 0.3%, lemongrass oil, 0.05% citral, and 0.1% citral, respectively. The experiments were conducted in triplicate.

[a] The reduction was statistically significant ($P \leq 0.05$) in comparison to the control (with no antimicrobial).

[b] Reductions were significantly different ($P \leq 0.05$) between the two concentrations of each antimicrobial.

[c] Reductions were significantly different ($P \leq 0.05$) between the comparable concentrations (i.e., low versus low and high versus high concentration) of the essential oil and its active component.

Table 2. Reduction (Log_{10} ± standard deviation)* in the population of *Escherichia coli* after exposure to various concentrations (vol/vol) of cinnamon oil or its active component, cinnamaldehyde in PBS.

Time (min)	Cinnamon Oil		Cinnamaldehyde	
	0.8 %	1.25 %	0.1 %	0.2 %
1	2.20 ^{ab} ± 0.07	> 5.53 ^{abc} ± 0.00	ND	0.69 ^{ac} ± 0.04
5	> 6.15 ^{ac} ± 0.00	> 5.53 ^{ac} ± 0.00	0.01 ^{bc} ± 0.07	3.01 ^{abc} ± 0.40
10	> 6.15 ^{a} ± 0.00	> 5.53 ^{a} ± 0.00	ND	5.11 ^{a} ± 0.91
15	> 6.15 ^{ac} ± 0.00	> 5.53 ^{a} ± 0.00	2.01 ^{ac} ± 0.58	ND
30	> 6.15 ^{a} ± 0.00	> 5.53 ^{a} ± 0.00	4.92 ^{a} ± 0.62	> 5.64 ^{a} ± 0.00

ND Not determined.

* Inoculated with 8.9×10^7 CFU/mL, 1.7×10^7 CFU/mL, 2.4×10^7 CFU/mL, and 2.4×10^7 CFU/mL for the experiment with 0.8% cinnamon oil, 1.25%, cinnamon oil, 0.1% cinnamaldehyde, and 0.2% cinnamaldehyde, respectively. The experiments were conducted in triplicate.

a The reduction was statistically significant ($P \leq 0.05$) in comparison to the control (with no antimicrobial).

b Reductions were significantly different ($P \leq 0.05$) between the two concentrations of each antimicrobial.

c Reductions were significantly different ($P \leq 0.05$) between the comparable concentrations (i.e., low versus low and high versus high concentration) of the essential oil and its active component.

Table 3. Reduction (Log_{10} ± standard deviation)* in the populations of *Escherichia coli* after exposure to various concentrations (vol/vol) of oregano oil or its active component, carvacrol in PBS.

Time (min)	Oregano Oil		Carvacrol	
	0.02 %	0.05 %	0.02 %	0.04 %
1	0.00 ± 0.05	0.32 ± 0.53	ND	ND
5	0.10 [bc] ± 0.09	> 5.35 [ab] ± 0.00	0.48 [abc] ± 0.10	> 5.67 [ab] ± 0.00
10	0.36 [bc] ± 0.28	> 5.35 [ab] ± 0.00	0.98 [abc] ± 0.05	> 5.67 [ab] ± 0.00
20	0.72 [ab] ± 0.38	> 5.35 [ab] ± 0.00	ND	ND
30	1.00 [abc] ± 0.49	> 5.35 [ab] ± 0.00	2.27 [abc] ± 0.22	> 5.67 [ab] ± 0.00

ND Not determined.

* Inoculated with 1.3×10^7 CFU/mL, 1.1×10^7 CFU/mL, 2.4×10^7 CFU/mL, and 2.4×10^7 CFU/mL for the experiment with 0.02% oregano oil, 0.05%, oregano oil, 0.02% carvacrol, and 0.04% carvacrol, respectively. The experiments were conducted in triplicate.

[a] The reduction was statistically significant ($P \leq 0.05$) in comparison to the control (with no antimicrobial).

[b] Reductions were significantly different ($P \leq 0.05$) between the two concentrations of each antimicrobial.

[c] Reductions were significantly different ($P \leq 0.05$) between the comparable concentrations (i.e., low versus low and high versus high concentration) of the essential oil and its active component.

Table 4. Reduction (Log_{10} ± standard deviation)* in the population of *Escherichia coli* after exposure to various concentrations of clove bud oil (vol/vol) in PBS.

Time (min)	Clove Bud Oil		
	0.06 %	0.5 %	1.0%
1	0.00 [a] ± 0.08	> 5.52 †[b] ± 0.00	> 5.45 †[b] ± 0.00
5	0.32 †[a] ± 0.10	> 5.52 †[b] ± 0.00	> 5.45 †[b] ± 0.00
10	1.20 †[a] ± 0.26	> 5.52 †[b] ± 0.00	> 5.45 †[b] ± 0.00
20	2.85 †[a] ± 0.68	> 5.52 †[b] ± 0.00	> 5.45 †[b] ± 0.00
30	> 4.34 †[a] ± 0.98	> 5.52 †[a] ± 0.00	> 5.45 †[a] ± 0.00

* Inoculated with 1.3×10^7 CFU/mL, 2.3×10^7 CFU/mL, and 2.3×10^7 CFU/mL for the experiment with 0.06%, 0.5%, and 1.0% clove bud oil, respectively. The experiments were conducted in triplicate.

† Reduction was statistically significant ($P \leq 0.05$) in comparison to the control (with no antimicrobial).

[a, b] Statistically significant ($P \leq 0.05$) differences per exposure time for clove bud oil concentration (within a row) are indicated by different letters (a or b). The same letter within a row indicates that there was no significant difference between the reductions observed between the different concentrations (e.g., at 30 minutes). Differing letters within the same row indicate that the reductions were statistically different (e.g., at 1 minute).

Table 5. Reduction (Log_{10} ± standard deviation)* in the population of *Escherichia coli* after exposure to two different concentrations (vol/vol) of allspice oil in PBS.

Time (min)	Allspice Oil	
	0.5%	1.0%
1	0.00 [b] ± 0.16	> 5.52 [ab] ± 0.00
5	0.00 [b] ± 0.05	> 5.52 [ab] ± 0.00
10	0.00 [b] ± 0.17	> 5.52 [ab] ± 0.00
20	0.00 [b] ± 0.08	> 5.52 [ab] ± 0.00
30	0.00 [b] ± 0.04	> 5.52 [ab] ± 0.00

* Inoculated with 6.6×10^6 CFU/mL and 2.3×10^7 CFU/mL for the experiment with 0.5% and 1.0% allspice oil, respectively. The experiments were conducted in triplicate.

[a] Reduction was statistically significant ($P \leq 0.05$) in comparison to the control (with no antimicrobial).

[b] Reductions were significantly different ($P \leq 0.05$) between the two different allspice oil concentrations.

Table 6. Reduction (Log_{10} ± standard deviation)* in the population of *Escherichia coli* after exposure to two different concentrations (wt/vol) of olive extract in PBS.

Time (min)	Olive Extract	
	1.0%	2.5%
15	1.02 [a] ± 0.15	1.22 [a] ± 0.25
20	2.29 [a] ± 0.11	2.24 [a] ± 0.12
25	2.73 [ab] ± 0.11	3.39 [ab] ± 0.15
30	3.08 [ab] ± 0.01	4.81 [ab] ± 0.23

* Inoculated with 1.5×10^7 CFU/mL and 1.2×10^7 CFU/mL for the experiment with 1.0% and 2.5% olive extract, respectively. The experiments were conducted in triplicate.

[a] Reduction was statistically significant ($P \leq 0.05$) in comparison to the control (with no antimicrobial).

[b] Reductions were significantly different ($P \leq 0.05$) between the two different olive extract concentrations.

Table 7. Reduction (Log_{10} ± standard deviation)* in the population of *Escherichia coli* after exposure to 6.0% green tea extract (wt/vol) or 5.0% grape seed extract (wt/vol) in PBS.

Time (hours)	Green Tea Extract	Grape Seed Extract
1	ND	0.00 ± 0.01
4	0.00 ± 0.08	0.00 ± 0.05
6	0.00 ± 0.00	0.03 ± 0.09
24	1.93 [ab] ± 0.03	> 5.05 [ab] ± 0.00

ND Not determined.

* Inoculated with 4.0×10^7 CFU/mL and 1.8×10^7 CFU/mL for the experiment with green tea extract and grape seed extract, respectively. The experiments were conducted in triplicate.

[a] Reduction was statistically significant ($P \leq 0.05$) in comparison to the control (with no antimicrobial).

[b] Reductions were significantly different ($P \leq 0.05$) between the green tea extract and grape seed extract experiments.

Table 8. Concentration and time required for plant-derived antimicrobials to achieve a 2.0-$\log_{10}$ or 99% reduction (T_{99}) in *Escherichia coli*.

Antimicrobial	Type	Concentration (%)	T_{99} (min)
Clove Bud Oil	Essential Oil	0.06	20
		0.5*	1
		1.0	1
Allspice Oil	Essential Oil	0..5	> 30
		1.0*	1
Cinnamon Oil	Essential Oil	0.8*	1
		1.25	1
Carvacrol	Active Component	0.02	30
		0.04*	5
Oregano Oil	Essential Oil	0.02	> 30
		0.05*	5
Lemongrass Oil	Essential Oil	0.05	10
		0.3*	5
Citral	Active Component	0.05	10
		0.1*	5
Cinnamaldehyde	Active Component	0.1	15
		0.2*	5
Olive Extract	Extract	1.0*	20
		2.5	20
Green Tea Extract	Extract	6.0*	> 1440
Grape Seed Extract	Extract	5.0*	1,440

* Lowest recommended concentration based on the experimental results to provide a rapid reduction in *E. coli* numbers.